

Amendments to the Specification:

1. Please insert new paragraphs [0010.1] through [10.21] followed by a new section heading into the specification at page 3:

[0010.1] Figure 1A is an electron micrograph prepared using transmission electron microscopy (TEM) of a type 1 proteon.

[0010.2] Figure 1B is a TEM electron micrograph of another type 1 proteon.

[0010.3] Figure 1C is a TEM electron micrograph of a type 2 proteon.

[0010.4] Figure 1D is a TEM electron micrograph of another type 2 proteon.

[0010.5] Figure 1E is a TEM electron micrograph of a negatively stained type 2 proteon.

[0010.6] Figure 1F is a TEM electron micrograph of another negatively stained type 2 proteon.

[0010.7] Figure 1G is an electron micrograph prepared using scanning electron microscopy (SEM) of a type 2 proteon.

[0010.8] Figure 2A is a graph illustrating proteon proliferation in purified water and in blood plasma showing the number of proteons visible as a function of time.

[0010.9] Figure 2B is a graph of proteon proliferation in two different growth mediums showing the number of proteons visible as a function of time.

[0010.10] Figure 2C is a graph of proteon proliferation in the presence and absence of urea showing the number of proteons visible as a function of urea concentration.

[0010.11] Figure 2D is a graph illustrating cyclic amplification of proteons in plasma showing the number of proteons visible as a function of the number of amplification cycles.

[0010.12] Figure 3A is a TEM electron micrograph of clusters of crystalline, metallic copper nanoparticles.

[0010.13] Figure 3B is a TEM electron micrograph of an amorphous matrix for the copper nanoparticles shown in Figure 3A.

[0010.14] Figure 3C is an SAD pattern identifying the copper nanoparticles in Figure 3A.

[0010.15] Figure 3D is a DF image of copper nanoparticles prepared using a portion of the FCC ring of Figure 3C.

[0010.16] Figure 4A is a three-dimensional bar graph illustrating the viability of three different cultured cells after exposure to proteon nucleation centers (PNCs) obtained from shark blood at four different concentrations of PNC/ml.

[0010.17] Figure 4B1 shows a dark field photomicrograph of brain glioma cells before exposure to PNCs.

[0010.18] Figure 4B2 shows a dark field photomicrograph of brain glioma cells after exposure to PNCs.

[0010.19] Figure 4B3 is a dark field photomicrograph of brain glioma cells after exposure to staurosporine.

[0010.20] Figure 4B4 is a fluorescence photomicrograph of brain glioma cells exposed to PNCs and stained with propidium iodide.

[0010.21] Figure 4C is a graph illustrating the viability of brain glioma cells obtained from various animals after exposure to PNCs at different concentrations.

DETAILED DESCRIPTION OF THE INVENTION

2. Please delete the subtitle DETAILED DESCRIPTION OF THE INVENTION currently found on page 5 of the specification after paragraph [0013] and before paragraph [0014]

3. Please replace paragraph [0054] with the following amended paragraph:

[0054] A 30 µl sample of freshly drawn full blood from a healthy human male was obtained and diluted in 1000 µl of purified water (~~Direct QTM, Millipore™,~~ (17 MOhm) in a 1.5-ml plastic vial, and centrifuged at 2200 g (6000 rpm) to obtain plasma. An additional aliquot of the blood was taken and prepared for optical dark-field microscopy. The plasma was transferred to a 4-ml glass vial with a plastic cap and a teflon liner. An aliquot of plasma was again retained for dark-field microscopy. The vial and contents were subjected to heating at a temperature of 120°C and 20 psi of pressure for two hours. An aliquot of the heat-treated plasma was prepared for dark-field microscopy.

4. Please replace paragraph [0067] with the following amended paragraph:

[0067] The quantitative analysis of proteins in the proteon samples was carried out by two different protein assays obtained from ~~BIO-RAD™~~ Bio-Rad Laboratories and ~~SIGMA™~~ Sigma Chemical Company according to the manufacturers' protocols. Samples of blood as described in the Example 1 were exposed to different temperatures and pressures. Results of the experiments are shown in Table 4.

5. Please replace paragraph [0072] with the following amended paragraph:

[0072] An aliquot of blood pre- and post-treatment (see Example 1) was purified using the ~~DNeasy Tissue Kit™~~ DNeasy™ genomic DNA isolation tissue kit (Qiagen) according to the manufacturer's standard protocols for blood and bacteria. After final elution, samples were loaded on 1% agarose gel. SNA bands were visualized with ethidium bromide. DNA was detected in pre-treatment sample and undetected in the post-treatment sample.

6. Please replace paragraph [0073] with the following amended paragraph:

[0073] Similar results were obtained using a High Pure PCR Template Preparation Kit™ from Roche for isolation of Nucleic Acids followed by fluorometric quantitation of double-stranded DNA using the ~~PicoGreen-dsDNA Quantitation Reagent™~~ PicoGreen® dsDNA quantitation reagent from Molecular Probes and a TECAN Spectrafluor Plus equipped with DeltaSOFT software for detecting fluorescence (excitation at 485 nm and emission at 535 nm). See Table 4.

7. Please replace paragraph [0083] with the following amended paragraph:

[0083] Optical observation of proteons was performed with an Olympus™ microscope fitted with a 100-W mercury lamp illumination source, a polarizer, a Naessens dark-field condenser (COSE Corp., Canada) and a 100x. objective (oil, NA 1.4). The dark-field images were directed to a ~~DEI-470T Optronics CCD Video Camera System™~~ DEI-470T™ microscope video camera (Optronics Engineering, CA) utilizing the methods described in Vodyanoy et al. (1994) Langmuir 10:1354-1357. A direct count of proteons was used to determine their concentrations in liquid samples, and Image Pro™ (Creative Software, Inc.) was used to quantify the number of proteons.

8. Please replace paragraph [0087] with the following amended paragraph:

[0087] Samples of proteons grown at the suppressive presence of 8 M Urea were subjected to dialysis using a ~~Pierce Slide-A-Lyser 10K™~~ Slide-A-Lyser™ 10K dialysis cassette (20 h, 5 L, 20°C) according to the manufacturer's instructions. The number of proteons found by dark-field microscope increased significantly. Samples of plasma treated with 120°C heat and 20 psi pressure and urea at concentrations of 0.01-8 M were taken. Polyacrylamide gel electrophoresis was carried out on each sample with a

4-20 % Tris-HCl Ready Gel™, Bio-Rad Ready™ precast gel (Bio-Rad) according to the manufacturer's protocol. The control (proteons without chaotropic compounds) showed two characteristic bands of 14,400 and about 8,000 D. The experimental samples (proteons in the presence of a chaotropic compound) displayed a diffuse distribution of proteins or fragments of proteins with no sharp bands of proteins of the high molecular mass range. As the concentration of urea increased, the intensity of the diffusion staining decreased, and almost fully disappeared at the 8 M concentration of urea. The proteon sample displays a 14,400 D band that coincides with a similar band found in plasma. When guanidine hydrochloride or urea was added to proteons produced without the chaotropic compounds, heat of 120°C and pressure of 20 psi resulted also in a great reduction of number of proteons visible by dark-field microscopy. Dialysis of these samples restored the population of the proteons. Gel electrophoresis of proteons and plasma treated with 120°C heat and 20 psi pressure at the presence of urea, and then dialyzed, reveals two bands of 14,400 and about 8,000 D in all samples, including those before and after dialysis.

9. Please replace paragraph [0096] with the following amended paragraph:

[0096] The impact of PNCs upon viability of various cultured cells was investigated using the tetrazolium salt (MTT) cell proliferation assay. RG2 (mouse brain glioma), F98 (rat brain glioma), Hs683 (human brain glioma), CTX TNA2 (rat transfected astrocyte), H9c2[2-1] (rat heart myocardium), 27FR (rat skin fibroblast), and SVGp12 (human brain astroglia) cells were obtained from American Type Culture Collection (~~ATCC™~~ATCC) and maintained as recommended by ATCC. MTT cell proliferation assays are commercially available. See, e.g., MTT Cell Proliferation Assay cell proliferation assay from ATCC™ATCC.

10. Please replace paragraph [0097] with the following amended paragraph:

[0097] Cells were plated in ~~D5648 (Sigma™)~~ Dulbecco's modified Eagle's growth medium (D5648, Sigma Chemical, St. Louis, MO) +10% FCS (~~HyClone™~~) fetal bone serum (FBS) (Hyclone Laboratories, Logan, UT) in polystyrene 96-well plates at a density 3×10^3 cells per well. Twenty-four hours after plating, the medium was replaced with DMEM with either staurosporine (100μl, 1μM) or PNC (aliquots, 100μl, 5×10^9 - 3×10^{11} PNC/ml). PNCs were isolated from blood obtained from shark, dog, and rabbit using the ultrafiltration protocol described in Example 5. PNCs were autoclaved at 120°C and 20 psi for 15 minutes before adding to the cell cultures.

11. Please replace paragraph [0098] with the following amended paragraph:

[0098] After 20 hours of treatment, a 20-μl aliquot of tetrazolium salt (MTT, 5 mg/ml in PBS) was added to the wells, and interaction was allowed to proceed for 4 hours at 37°C. MTT was reduced in metabolically active cells to form purple formazan crystals, which were dissolved by DMSO and quantified by a Bio-Rad™ plate reader (~~BioRad™~~). For each cell type, a linear relationship between cell number and absorbance is established, enabling accurate, straightforward quantification of changes in proliferation.

12. Please replace paragraph [0121] with the following amended paragraph:

[0121] Experiments were carried out to compare the proteons obtained by the procedure described in the Example 1 with the nanobacteria isolated from blood by Kajander et al. (1996) Mol. Biol. Cell 7:3007-3007 using the Nanocapture-ELISA™, an Enzyme-Linked Immunosorbent Assay, Nanocapture™ enzyme-linked immunosorbent assay (ELISA) kit for detection of nanobacterial antigens (Nanobac OY, Finland). The

Application No. 10/674,750

Amendment Dated August 3, 2005

Reply to the Final Rejection in the Office Action of April 20, 2005

nanobacteria included in the Nanocapture™ ELISA kit were used as a positive control. The Bio-Rad Microplate Manager™ 4.01 Bio-Rad software was used to obtain the results of the ELISA. The assay procedure was carried out following the manufacturer's recommendations. The reaction was considered to be positive when the absorbance was significantly higher than the level of noise. Results of interaction of antibodies grown against nanobacteria with proteons and plasma are summarized in Table 11.